10 µg of oligo-dT primer (Takara Shuzo) and 20 µl of water treated with diethylpyrocarbonate (DEPC, Wako Pure Chemical Industries).

Reaction mixture B: 12 µl of 5 x AMV RTase buffer (Life Science), 0.5 mM each of dATP, dCTP and dGTP, 0.2 mM dTTP, 60 U of RNase inhibitor (Takara Shuzo) and 0.1 mM Cy3-labeled dUTP (Amersham Pharmacia).

The reaction mixture A was incubated at 70°C for 10 minutes and then cooled on ice. The reaction mixture B was added thereto, and the resulting mixture was incubated at 42°C for 5 minutes. About 60 U of AMV RTase (Life Science) was added thereto. DEPC-treated water was further added to make the final volume to 60 µl. The resulting RT reaction mixture was incubated at 42°C for 70 minutes. 7.5 µl of 500 mM EDTA solution and 15 µl of 1 M sodium hydroxide were added to the reaction mixture. The mixture was incubated at 60°C for 1 hour to degrade the template RNA. After cooling to room temperature, 37.5 µl of 1 M tris-hydrochloride (pH 7.5) was added to the mixture. The solution was concentrated to 20 µl using Microcon YM-30 (Millipore), 200 µl of 10 mM tris-hydrochloride containing 1 mM EDTA was added thereto, and the resulting mixture was then concentrated to 20 µl. The thus obtained Cy3-labeled cDNA solution was used for the subsequent hybridization.

(5) The Cy3-labeled cDNA as prepared in (4) above

was heat-denatured, and the whole solution was dropped onto the DNA array as prepared in (1) above. A cover glass was placed on the spotted solution and the sides of the cover glass were sealed with a film. After incubation at 40-45°C for 10 hours, the cover glass was removed. The DNA array was washed in 0.2 x SSC/0.1% SDS for 30 minutes and in 0.2 x SSC for 30 minutes, and then air-dried. The fluorescent signals from the respective spots on the DNA array were analyzed using a microarray scanner (GMS). Representative values each obtained by dividing a fluorescence signal value for a sample treated with one of the substances by a fluorescence signal value for a control sample are shown in Table 5. In Table 5, a value greater than 1.00 indicates that the gene expression is promoted by the treatment with the substance. A value smaller than 1.00 indicates that the gene expression is suppressed by the treatment with the substance. A value equal to 1.00 indicates that the gene is not influenced by the test substance.

Table 5

Immobilized gene (Gene product)	DES treatment 2hr / 24hr	BisA treatment 2hr / 24hr	E_2 treatment 2hr / 24hr
Nuclear receptor or nuclear receptor transcriptional coupling			
p300/CBP	1.28 / 4.45	1.50 / 1.07	1.67 / 3.39
N-CoR/SMRT	0.62 / 1.42	1.17 / 1.08	1.86 / 1.51
ACTR	1.14 / 4.97	0.47 / 1.03	1.19 / 3.27
RIP 140	1.74 / 2.51	1.70 / 1.14	1.46 / 2.34
TIF2	1.19 / 3.04	2.66 / 0.84	2.17 / 3.30
ARA 70	0.63 / 1.37	1.31 / 0.93	1.47 / 1.45
Kinase-type signal transduction			
JNK2	0.72 / 1.85	1.30 / 0.71	1.86 / 1.71
BMK-2	1.15 / 6.06	1.04 / 0.29	1.13 / 0.05
Oncogenes			
c-Myc-1	1.08 / 0.00	1.40 / 1.30	2.52 / 1.89
Bax	1.34 / 2.27	2.41 / 1.44	1.32 / 0.99
Receptor-type kinase			
PDGF receptor	0.65 / 3.04	1.25 / 1.19	1.63 / 2.89
VEGF receptor	1.15 / 3.27	0.37 / 0.46	2.13 / 2.94

In many cases, abnormal reproduction in wild animals and reduced spermatogenesis in humans presumably caused by disruption by endocrine disrupting activities are considered to be due to suppression or interruption of signals for endocrine action at a certain stage. Therefore, it is considered that genes of which the expression is suppressed when compared with the expression in control cells should be noticed in addition to overexpressed genes. For example, among the genes used in this example, promotion of the expression of many genes that are